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A Comparison of Methods for Assessing the Amount of Fungal Mycelium in Soil Samples

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Synopsis

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In this investigation a series of comparative tests were conducted to assess the relative values of soil sectioning, the agar film technique, the soil suspension slide method, and the impression slide technique in affording a means for assessing the amount of fungus mycelium in soil.

It was apparent that, for simple comparative assessments of the mycelium concentration of different soils, the measurement of percentage frequency of occurrence of mycelium on sampling fields of known size, based on the microscopic observation of impression slides, afforded satisfactory data without excessive labour.

For an absolute measure of mycelium concentration, the measurement of mycelial lengths by the agar film technique was found to be the most accurate.

The major value of the soil sectioning technique appeared to be that it permitted microscopic examination of soil in an undisturbed state, and could provide information on fungal growth patterns and substrate relationships in soil.

Im Rahmen dieser Untersuchung wurden eine Reihe von vergleichenden Tests durchgeführt, um den relativen Wert verschiedener Methoden abzuschätzen, mit deren Hilfe man Schätzungen der Menge von Pilzmyzel im Boden erlangen kann (Bodenschnitte, Agarfilm-Technik, Objektträger-Bodensuspension- und Objektträger-Abdruck-Methode).

Es ist augenscheinlich, daß (für einfache vergleichende Schätzungen der Myzelkonzentration in verschiedenen Böden) Messungen der prozentischen Häufigkeit des Vorkommens von Myzel (auf Untersuchungsflächen bekannter Größe), die sich auf mikroskopischen Befunden von Abdruck-Objektträgern gründen, befriedigende Daten ohne übermäßige Arbeit ergeben.

Für eine absolute Messung der Myzelkonzentration wurde die mit Hilfe der Agarfilm-Technik durchgeführte Messung der Myzellänge für am meisten akkurat befunden.

Der Hauptwert der Bodenschnitt-Technik schien zu sein, daß sie eine mikroskopische Durchmusterung von Boden in ungestörtem Zustand erlaubt und daß sie über die pilzlichen Wachstumsformen sowie über die Substratbeziehungen der Pilze in Böden Aufschluß geben könnte.

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1. Review of available techniques

The morphological form of fungi in soil, with their mycelial and spore components, together with the diversity in their patterns of growth have imposed many difficulties as regards their study. The realization of these difficulties has led to the development of a range of methods for studying soil fungi, methods which, in the main, can be grouped as either those involving the isolation of fungi from soil or those involving the microscopic examination of fungi in the soil.

In attempts to assay the amount of fungus material in soil many workers have resorted to counts obtained by use of the soil dilution plate technique. A great deal has already been written in criticism of this use of the soil dilution plate technique, and all that need be said here is that the concept of counting individual fungi (as is done for the soil bacteria) is inadmissible — the expression "individual fungus" having no real meaning (BURGES, 1953) because of the hyphal growth patterns of most fungi. The counts obtained from soil dilution plates allow the assaying of the spore content of soil but do not allow one to obtain any idea of the amount of fungus mycelium in a given weight or volume of that soil. It is this latter information which is often of prime importance to investigators of biomass or microbial activity in soil.

The suggestion that fungi were not active in soil led to both the indirect and direct proof that mycelial development in the soil did in fact occur. WAKSMAN (1916) demonstrated by cultural techniques that mycelial growth took place and CONN (1922) provided direct evidence of the presence of fungal hyphae in dried and stained soil smears. This work of CONN may be considered the effective basis of all visual examination techniques which have subsequently been developed — the new techniques generally attempting to put data on a quantitative basis or to allow the direct observation of undisturbed soil.

ROSSI (1928) pressed clean microscope slides against exposed soil surfaces and after heating, stained the soil preparations to display the fungi present in the soil. This technique has been variously modified by the use of slides coated with agar or gelatin (ROSSI, RICARDO, GESUE, STANGANELLI and WANG 1936) or nitrocellulose (BROWN, 1958). However such procedures, as also those of CONN (1922) and WINOGRADSKY (1925), all suffered from the same disadvantage — they could not give an accurate, absolute measure of the amount of mycelium present in the soil because of the difficulty of accurately determining the exact quantity of soil examined. This difficulty was soon recognized and attempts made to overcome it were made by CONN (1918) who used the technique of spreading 0.1 ml of soil suspension of known dilution over 1 sq. cm of microscope slide. WINOGRADSKY (1925) attempted to estimate the quantity of soil observed by measuring the increase in weight of the slide on which the soil smear had been fixed. However, THORNTON and GRAY (1934) pointed to the inherent inaccuracies involved in both these methods due to the micromasurement of volume and weight respectively. Moreover they pointed out that the calculation of the number of organisms per unit amount of soil involves an estimate not only of the small amount of soil in the film, but also of the number of organisms in that film. They showed that the latter estimate is also liable to be a great source of error since it is based on the mean count of a number of random microscopic fields, and consequently its reliability depends on the assumption that the distribution of organisms over the film is random. Statistical analyses of such counts, obtained by KUHLMORGEN-HILLE (1928) and themselves (THORNTON and GRAY, 1934) lead them to the conclusion that bacteria in a dried film of soil are distributed so unevenly that direct counts in random throws do not provide valid samples of the whole film, and therefore do not provide reliable data from which to calculate the total bacterial contents of the soil films.

In an attempt to overcome this difficulty THORNTON and GRAY (1934) devised a counting procedure based on a ratio method in which a known quantity of soil is added to a

counted suspension of indigo particles of a size and density approximately similar to that of the bacteria. Although the surface tension forces operating during drying of the soil film alter the distribution of the bacteria it was considered that the indigo particles would be similarly affected. From the observation of the ratio of bacterial numbers: indigo particles in a definite number of fields it is possible to estimate the numbers of bacteria per unit amount of soil.

The techniques described by CONN (1918), WINOGRADSKY (1925) and THORNTON and GRAY (1934) were mainly devised for the enumeration of the bacterial populations by direct counting of the number of individuals present in the soil, and are of limited value for the study of fungal populations. As has been stated earlier, the expression individual fungus has no real meaning in the sense that we may talk of individual bacteria or soil animals. Moreover the improved technique of the ratio method would be very difficult to apply to the study of fungal populations since no suitable contrasting ratio particle of similar shape and density of the fungus mycelium can be envisaged.

These difficulties were overcome by the development of the agar-film technique (JONES and MOLLISON, 1948), in which a homogeneous distribution of both organisms and soil particles could be obtained in a soil suspension in agar and the thin films subsequently prepared from it. The viscosity of the agar prevents soil flocculation, and instant gelatinization prevents disturbance of particles by surface tension forces during drying, and thus a count of known volume of the soil could be made when films were prepared on a haemocytometer slide of known depth. Statistical analysis of counts obtained by JONES and MOLLISON (1948) showed that random distribution of organisms had been achieved, and consequently the need for a ratio method of counting was obviated.

The visual examination techniques discussed so far have involved a marked disturbance of the soil during the preparations for subsequent observation — indeed some of these techniques deliberately aimed at such disturbance in order to obtain a homogeneous distribution of the soil organisms on the slide (THORNTON and GRAY, 1934; JONES and MOLLISON, 1948). In the impression slide technique (ROSSI, 1928) and its various modifications although a marked disturbance of the soil is effected, there is no attempt to bring about a homogeneous dispersion of the soil organisms. The only homogeneity aimed for has been that of a thin even film of soil of known amount over the slide by the use of different adhesive mixtures. Consequently with such impression techniques the quantitative assessments of fungal populations have been restricted to only simple comparative data and have not provided absolute measures of fungal development.

The severe disturbance of the soil necessary in the techniques described has the severe disadvantage that by their application no information on the growth patterns of fungi in soil can be obtained. If such information is required then it is vital that the procedures involved in the preparation of soil for visual examination should be such as to prevent any marked disturbance of the soil (and thus allow observation of the fungal populations "in situ").

KUBIENA (1938) demonstrated that a great deal of valuable information on soil micro-organisms can be obtained by the direct examinations of soil in the field using a Leitz Ultrapak. Such a technique has been shown to be of great value in both "in situ" and "in vivo" studies of fungal populations.

Although such a technique is theoretically ideal the practical difficulties and inconveniences involved have induced many workers to devise methods by which such examinations may be conducted under laboratory conditions. This has had the immediate effect that studies can no longer be conducted "in vivo", but the advancement in techniques and the quality of materials available have permitted the preparation of thin sections of soil in an undisturbed state thus following the observations of the soil fabric and micro-organisms "in situ".

The first thin sections of soil were prepared by DELAGE and LAGATU (1904), but in their technique the soil had to be crushed before being mixed with the hardening substances and consequently the natural fabric of the soil was destroyed. The first advance on such a technique was put forward by ROSS (1924) and KUBIENA (1938) — at first bakelite and Canada balsam were used as the impregnation agents, but both workers finally proposed the use of kolloid as being the most satisfactory material. With both techniques it was possible to carry out the satisfactory impregnation of a block of soil without first breaking up the soil. However the procedure contained the important defect that it was necessary to apply heat in the drying of the soil prior to impregnation and also during the subsequent hardening of the kolloid. This use of heat, especially before impregnation of the soil, can cause severe distortion of the soil structure (by uneven drying of its components) and shrinkage and displacement of the microorganisms.

With the realization of these deleterious effects of heat a number of techniques were developed which aimed at avoiding such distortion. Both HAARLØV and WEIS-FOGH (1953) who used agar-agar as the impregnation material, and MINDERMAN (1956) who used gelatin, obviated the necessity for preliminary drying of the soil prior to impregnation, since these materials both not fully miscible with water. Moreover subsequent hardening of the samples could be effected by treatment with 98 % alcohol and 10 % formalin respectively (thus again avoiding the application of heat).

Techniques which entail the use of natural or synthetic resins necessitate the drying of the soil prior to impregnation since these materials are totally immiscible with water. Consequently, where resins have been used as impregnating materials, workers have attempted to reduce the distortion caused by the drying procedure. Thus BOURBEAU and BERGER (1948), DAY (1949) and HEPPLE and BURGESS (1956) managed to get the soil sufficiently dry by air-drying at room temperature, thus limiting the amount of disturbance in comparison with the rapid drying in an oven. An important advance was the application of the freeze drying technique to the soil sample (ALEXANDER and JACKSON, 1955; BURGESS and NICHOLAS, 1961) — a technique which prevents any disturbance of the soil structure or microorganisms during the removal of water.

There has also been the development in the avoidance of heat disturbance during the hardening of the resins with the change in choice of materials from those requiring "curing" by heat (FRET, 1947; MOCHALOVA, 1956) to the use of synthetic polyester resins which set irreversibly under the actions of a catalyst. Several such resins have been used e.g. "Castolite" (BOURBEAU and BERGER, 1948; DAY, 1949), "Bakelite" (HEPPLE and BURGESS, 1956), "Polyester" (ALTEMÜLLER, 1956), "Maco" (ALEXANDER and JACKSON, 1955) and "Vestopal" (JONGERIUS and HEINTZBERGER, 1963). These latter materials, which harden under the action of a catalyst, allow avoidance of disturbance of the soil structure which might otherwise occur if direct heat is used during the "curing" process.

The production of thin soil sections of known thickness from rock hard resin-impregnated soil samples entails the use of standard geological procedures (cutting, grinding and polishing). However where softer impregnating agents such as agar-agar or gelatin have been used, the thin sections are obtained by sectioning a microtome (HAARLØV and WEIS-FOGH, 1953; MINDERMAN, 1956). Such a procedure severely limits the minimum thickness of sections which can be obtained — especially in mineral soils (where the microtome knife must either push out or in the mineral grains which contact the blade). MINDERMAN (1956) overcame this difficulty by soaking the gelatin impregnated soil sample in hydrofluoric acid in order to dissolve away the silica material. After a second impregnation in gelatin, the soil sample could be cut to any desired thickness.

The application of stains during the preparation of soil sections has sometimes been attempted. Where rock hard impregnated soil blocks are used it is necessary that stains be applied before impregnation with the hardening agent. ALEXANDER and JACKSON

(1958) attempted such a procedure, but later attempts to apply this refinement (BURGES and NICHOLAS, 1961) have shown a great disturbance of soil structure due to the staining and necessary subsequent washing procedures.

The techniques using agar-agar and gelatin as impregnating agents have the advantage that they allow staining of the soil microorganisms after the impregnation of the soil. Undoubtedly staining of the soil preparations facilitates the study of the soil microorganisms, making them more easily discernible within the soil fabric. Thus HAARLØV and WEIS-FOGH (1953) stained their sections with violamin, a vital stain first used by ROMELL (1934), whilst MINDERMAN (1956) applied the quadruple staining method of JOHANSEN (1940).

The past decade has seen the development of techniques which permit the preparation of thin sections of soil without any marked disturbance or distortion, thus allowing detailed investigations of the micromorphology of soil and studies on soil microorganisms *in situ*. However, little advance has been made on two other limitations to the use of soil sections for studies on populations of soil fungi. Firstly there still remains the general inability to identify the organisms seen in soil sections; secondly no satisfactory technique has been devised to allow the determination of the viability of these organisms. Thus although microscopic examination of soil sections permits a direct measurement of the amount of mycelium in a unit amount of soil, it is seldom possible to identify the organisms (except in the unfrequent event of fruiting structure being observed). It is therefore normally only possible to classify observed mycelia into crude morphological groups (NICHOLAS, PARKINSON and BURGES, 1964).

Similarly no real advance has been made regarding differentiation of viable and inviable mycelium. The use of vital staining techniques is somewhat restricted in that a negative result may be obtained not only through the inviability of the mycelium but also by the masking of the coloured vital stain by the natural dematiaceous colour of the mycelium, or through failure of penetration of the stain into the fungal protoplasm. The use of fluorescent staining techniques, although satisfactorily applied in studies of bacterial populations (STRUGGER, 1948) have not so far been successfully developed for mycological studies.

Although many techniques have been described for the preparation of undisturbed thin sections of soil the application of these soil biological studies has been very restricted (HAARLØV, 1955; BURGES and NICHOLAS, 1961; JONES and GRIFFITHS, 1964; NICHOLAS, PARKINSON and BURGES, 1964). This is undoubtedly a reflection of the amount of labour involved in such techniques in comparison with the expected return of data otherwise unobtainable.

The other visual examination techniques, which involve much less labour in preparation, since they do not attempt to maintain the undisturbed fabric of the soil, have been more widely used in microbiological studies (though usually only in the hands of those workers who developed them). Thus SKINNER, JONES and MOLLISON (1952) compared the values of direct and plate-counting techniques for the quantitative estimation of soil bacteria and fungi, using the agar film technique (JONES and MOLLISON, 1948) for the direct count. WITKAMP (1960) applied his soil suspension slide technique in a study of the amount of fungal mycelium in dune development. However, the lack of information regarding the practicability and applicability of the various techniques described, in the hands of workers other than those who developed them, is a most important omission as regards a critical evaluation of these techniques.

In the following section an attempt will be made to provide data assessing the relative merits of several of the techniques described above.

2. Value and limitations of several techniques

2.1. Prefatory note

In the course of the work reported here the value and limitations of four techniques for the microscopic examination of fungal mycelium present in soil were assayed. The techniques were:

1. Soil sectioning technique (BURGES and NICHOLAS, 1961),
2. Modified impression slide technique (BROWN, 1958),
3. Agar film technique (JONES and MOLLISON, 1948),
4. Soil suspension slide technique (WITKAMP, 1960).

The aim in the application of these techniques being to obtain information on the amount of fungal mycelium present in soil.

2.2. Details of the methods used

(1) Soil section technique

The procedures involved in the preparation of soil sections (collection of soil samples, fixing and staining, washing, drying, impregnation with resin, standard geological techniques of grinding, cutting, polishing and mounting) as used in this study have been described in detail elsewhere (BURGES and NICHOLAS, 1961; NICHOLAS, PARKINSON and BURGES, 1964).

(2) Modified impression slide technique

This technique, devised by BROWN (1958) as a modification of the technique of ROSSI and RICCARDO (1927), may be divided into the following distinct operations:

- a) Preparation of the adhesive mixture,
- b) Sampling of the soil,
- c) Fixation, staining and washing of the soil film.

a) Preparation of adhesive:

BROWN (1958) used a mixture of nitrocellulose in amylacetate and 5% castor oil, however in this investigation a colloidion — castor oil — amylacetate mixture (6 : 1 : 2 on a volume basis) was used.

b) Soil sampling:

After exposing a soil profile the exposed soil surface was cleaned and levelled using a sharp razor. The adhesive mixture was spread over an area of a $2\frac{1}{2} \times 1''$ clean glass slide by means of a thick camel-hair brush, and the slide was then pressed for 20 seconds against the soil surface.

In order to obtain comparable replicate slides it was important that the adhesive be spread thinly and evenly over the slide surface, and that only light pressure be used to obtain the impression. Any excess soil on the slide, not held by the adhesive mixture, could be removed by gently tapping the slide.

c) Fixation and staining:

The slides were stained for one hour in the combined fixative-staining solution of phenolic aniline blue, rinsed in distilled water, and left to dry at room temperature.

(3) Agar film technique

The technique used was similar to that proposed by JONES and MOLLISON (1948) with the slight modifications described by THOMAS, NICHOLAS and PARKINSON (1964).

(4) Soil suspension slide technique

The technique employed was similar to that proposed by WITKAMP (1960), with slight modifications.

After exposing a soil profile five samples of soil were collected from each horizon (using sterile sampling tubes). In the laboratory these five samples were sub-sampled (2—3 ccs from each sampling tube) and thoroughly mixed together in order to obtain a composite sample of soil (approximately 10—15 cc from each horizon).

Approximately 1 cc portions of this composite soil sample were then accurately weighed into each of 10 test tubes, and the rest of the soil was used for an accurate moisture content determination. Into each test tube 10 ml of a 5% aqueous resin (Celodal 1, Merck) was pipetted. The tubes were then shaken 20 times end over end, and allowed to settle for 10 seconds, after which a 0.5 ml aliquot was pipetted off from 1 cm below the surface on to a large ($3 \times 1\frac{1}{2}''$) microscope slide warmed over a water bath at 90°C. The 0.5 ml sample was spread over an area of approximately 15 sq cm and during the evaporation of the water the suspension was stirred with a needle in an attempt to prevent flocculation of the small mineral or humus coated particles.

In the procedure described by WITKAMP (1960) the initial soil sample for preparation of the suspension was based on weight and not on volume. However, in this study, as in the application of the other techniques the initial soil sample was taken on a volume basis. This was accurately weighed and all calculations for subsequent dilution correction were based on this weight, additional correction then being made for moisture content and "field density".

In addition to this modification, it was also decided to measure the surface area of the soil smear as considerable variation was noted between the preparations from the different soil horizons. This was then applied to the dilution factor as an additional correction factor.

3. Observations of the soil preparations

Within the range of techniques for the quantitative measure of vegetation, in higher plant ecology, two fundamentally different methods of approach are apparent (GREIG-SMITH, 1957).

Firstly, by applying techniques which give an absolute measure, where the result obtained is independent of the method used to determine it (within the limits of observational error). Within such techniques the more important and most frequently used methods of measure are:

- a) Density — the measure of the number of organisms per unit area.
- b) Cover — the proportion of the area occupied by the organism.
- c) Various methods of measuring yield — in which determinations of quantity of organisms per unit area are based on such data as dry weight or volume.

Secondly, by applying techniques which give a non-absolute measure, where the result obtained is dependent on the mode of sampling and only has meaning when coupled with a statement of the method used. The only techniques of any importance in this category are those involving a measurement of frequency (where the chance of finding the organism in a particular sample area is estimated).

The application of these methods to fungal populations of soil is fraught with difficulty. The mycelial form of fungi raises problems akin to, but greater than, those encountered in higher plant ecology. The actual pattern of growth varies a great deal between different fungal species (GARRETT, 1951; BURGESS, 1960). Thus, because of the form and pattern of growth of fungi, enumeration of fungal populations on the basis of particulate individuals per unit amount of soil is impossible. Microscopic examinations of soil preparations permits the observation of fungal hyphae fragments in a unit amount of soil. Such a count is not comparable with the normal density count (where the number of individual organisms per unit area is recorded) because of the form and pattern of growth of the soil fungi and because of the actual procedures involved in the preparation of the soil for microscopic observation.

The techniques which involve a mechanical disturbance to the soil sample, such as by grinding (JONES and MOLLISON, 1948), increase the number of pieces of hyphae by breaking up pre-existing individual lengths of mycelium into separate smaller pieces; the amount of destruction being largely dependent on the degree of grinding and the different rates of fragmentation of the various morphological groups of mycelium (SKINNER, JONES and MOLLISON, 1952). This is an extreme example, but even the milder forms of disturbance, such as the shaking in solution in the soil slide technique (WITKAMP, 1960), will have a similar, but lesser, effect. In the soil sectioning techniques, where there is little or no mechanical disturbance, it would be hoped that such an inaccuracy, due to the technique itself, would be overcome. However, in soil sections, of limited thickness, the mycelium observed is restricted to a very narrow three-dimensional range. The action of grinding to a particular thickness may result in an originally long piece of mycelium being separated into two or more distinct pieces. The degree of this inaccuracy will largely depend on the growth patterns of the various members of the fungal populations and the thickness of the section.

Because of this, depending on procedures involved in the preparation of the soil for observation, the so-called density count, based on the number of pieces of hyphae per unit amount of soil, is to be considered a non-absolute measure in determinations of soil fungal populations. The same could be said for soil actinomycetes (of the *Streptomyces* group), but with other groups of soil microorganisms (bacteria, protozoa, *Nocardia* group actinomycetes) it is reasonable to consider such a measure as being absolute.

The use of percentage cover as an absolute quantitative measure for soil fungal populations is not very practicable and little effort has been expended in this direction. The most popular technique in higher plant ecology involving some type of point quadrat would be of limited use, whilst the modification of the line transect (measuring the length of interception made by the different organisms) is only slightly better, as regards degree of effort in relation to information obtained. WITKAMP (1960) proposed the application of such a method, based on that of HENNIG (1958), whereby the number of intersections of the observed hyphae and the lines of an integration eyepiece are counted.

From the foregoing comments it will be apparent that the only satisfactory absolute measure of soil fungi must be based on some form of yield determination. GREIG-SMITH (1957) pointed out that relatively much less use had been made of such techniques in higher plant ecology, but their value in problems relating to humus or mineral nutrient turnover has not been overlooked (BLOW, 1955; BURGESS, 1956). Attempts to apply such measures to the soil fungal populations have only met with partial success. No technique has yet been developed which allows for the complete separation of the fungal mycelium from the soil, and consequently it is not yet possible to obtain information as to the dry-weight of fungal mycelium present in a unit amount of soil.

However, in most of the techniques involving microscopic examination of soil preparations, it has been possible to attempt a measurement of the length of fungal mycelium present in a unit amount of soil. Whilst length is not as good a measure as would be volume, the discrepancy between the two measures does not outweigh the advantage as far as labour of recording is concerned. It appears that the only suitable method of obtaining an absolute measure of fungal mycelium in the soil, with the techniques so far developed, will have to be based on the measurement of the length of fungal hyphae present in a unit amount of soil.

By far the most common methods for the quantitative assessment of fungal mycelium have been based on non-absolute measures (which have the advantage over absolute measures as far as the amount of labour involved is concerned, hence their popularity). The two major approaches have been that of percentage frequency of occurrence and of density.

The limitation of measures based on density of fungal mycelium have already been discussed. Percentage frequency of occurrence has been most widely used because of its speed and simplicity, but this method of measurement suffers from the same limitations as density (as far as mechanical disturbance of the soil is concerned). Moreover this assessment, based only on presence or absence of mycelium, is far less sensitive than that of density (where the number of hyphal units are counted). Further, percentage frequency, being a non-absolute measure in the true sense of the definition in that the results only have meaning when coupled with a statement of the sample area used, is of even less value as regards the comparison of results obtained by different workers using various sample areas.

Thus it is apparent that with the visual examination techniques so far developed there are three major methods for the quantitative assessment of fungal mycelium in the soil:

- a) percentage frequency of occurrence, in which the results are not expressed in relation to a unit amount of soil. Here the result is dependent on the method of observation (size of sample area) and on the method of preparation of the soil for observation i.e. a non-absolute measure.

- b) density of hyphal units, in which the results are expressed in relation to a unit amount of soil. However, although the results are independent of the method of observation they are much dependent on the procedures involved in the preparation of the soil for observation i.e. a non-absolute measure.
- c) length of fungal mycelium, in which the results are expressed in relation to a unit amount of soil. The results obtained are independent of the method of observation and (theoretically) independent of the method of preparation of the soil for observation i.e. an absolute measure. However, it should be remembered that the amount of mycelium observed may very well vary because of the ease of its observation, which itself may be greatly affected by the procedures involved in the preparation of the soil e.g. staining, disruption of the soil fabric, etc.

In the present study these three principal methods of quantitative assessment were applied, wherever possible, to the four techniques of visual examination employed. In the case of the modified impression slide technique, where it was impossible to relate observation to a unit amount of soil, there was no value in attempting measurements of hyphal length. Such measurements were made in the application of the other three techniques.

Also in the modified impression slide technique and the soil suspension slide technique, in which uncovered preparations were obtained, it was not easy to distinguish between separate hyphal units. Consequently measurement of density was restricted to the other two techniques.

The measurement of percentage frequency of occurrence only has meaning in relation to the particular size and shape of the sampling area. Hence it was essential to employ a standard sampling area during this study. The determination of this sampling area was made using the soil sectioning technique and then applied to the other techniques as well.

After a series of preliminary observations it was concluded that the size of the sampling area must be relatively large in comparison with the abundance of mycelium present. Circular sampling areas (fields) were considered preferable to square fields because of the smaller edge-effect for any given area.

The choice of a field size which would be suitable for a soil horizon with abundant fungal development would not be so suitable for a lower, more barren, soil horizon. In this study a compromise was attempted — using a circular field, 460 μm in diameter.

The comparative assessment of the value of these visual examination techniques was made using soil samples from the horizons of the Delamere Forest podzol (WILLIAMS and PARKINSON, 1964; NICHOLAS, PARKINSON and BURGESS, 1965).

When the agar film techniques and the soil suspension slide technique were applied in measuring the percentage frequency of occurrence of mycelium it was found that for the A_0 H, A_1 and A_2 horizons values of over 90% were obtained. Consequently such data are not given for these techniques as it was apparent that the field size (460 μm diameter) was too large to allow this method of measure to be of any critical value when applied with these two techniques. Clearly this method of measure could be made more critical for these techniques by simply reducing the field area used, however if this were done for these techniques the data obtained would no longer be comparable with those obtained using the modified impression slide technique and soil sections.

It was invariably found that results obtained from the B_2 and C horizons were dependent on the soil sampling procedure — which seems to have been insufficient for these horizons (relatively low in mycelium content as compared with the overlying horizons). Hence little attention will be paid to the data obtained from the B_2 and C horizons.

4. Comparative tests of the four visual examination techniques

4.1. Percentage frequency of occurrence of mycelium in circular fields, 460 μm diameter

This method of measure was applied, using the soil sectioning technique and the impression slide technique, to the assessment of the amount of mycelium in the different podzol horizons at bi-monthly intervals for one year.

For the soil sectioning technique, data were obtained by observing 5 or 6 slides per horizon at each sampling time (200 observations being made per slide). For the impression slide technique, ten slides were prepared from each horizon at each sampling time, and for subsequent observation 5 replicate slides were chosen (200 observations being made per slide).

The minimum and maximum values obtained by both techniques, together with the calculated maximum : minimum ratios, are given for each horizon in Table 1. Comparison of the maximum : minimum values shows that in every horizon there is less fluctuation in the figures obtained from observation of impression slides than in those obtained from soil sections. Thus the measure of percentage frequency of occurrence based on impression slides is a less sensitive measure of fluctuation in the amount of mycelium in the soil than is the same measure based on observation of soil sections.

Table 1 Comparison of the percentage frequency of occurrence data obtained from observations of soil sections and impression slides. Check on the sensitivity of the two techniques

Soil horizon	Soil sections			Impression slides		
	Minimum	Maximum	Max./Min.	Minimum	Maximum	Max./Min.
A ₀ H	49.2	71.5	1.46	59.0	85.0	1.44
A ₁	29.9	79.7	2.67	49.6	86.4	1.74
A ₂	21.4	68.4	3.20	41.0	75.8	1.85
B ₁	19.0	47.9	2.52	32.2	53.8	1.67
B ₂	5.2	18.0	3.50	16.2	30.0	1.85
C	1.3	4.4	3.32	6.4	19.2	3.00

The measure of percentage frequency of occurrence of mycelium was usually markedly higher on impression slides than on soil sections. This is shown in Table 2, which gives the overall mean percentage frequencies of occurrence of mycelium in each horizon (based on the six periodic samples) obtained by both techniques. The degree of increase in the figures from impression slides over those from soil sections is indicated by the simple ratio of their respective values.

Table 2 Comparison of the mean percentage frequency of occurrence data obtained from observations of soil sections and impression slides (based on 6 periodic samples)

	Soil horizon	Soil sections	Impression slides	Impression slides
				Soil sections
	A ₀ H	62.22	77.66	1.24
	A ₁	57.73	70.23	1.22
	A ₂	46.41	57.46	1.24
	B ₁	34.22	44.16	1.29
	B ₂	10.35	24.66	2.40
	C	2.47	12.03	4.85

The higher figures obtained from the impression slides are likely to be the major factor in reducing the sensitivity of the measure of percentage frequency obtained by this technique as compared with similar measures obtained from soil sections.

With both techniques the sensitivity of measure of percentage frequency of occurrence of mycelium could be increased by a reduction in the area of the chosen field — this giving a lower value for the horizons with a high concentration of mycelium. But reduction in the size of the field would also mean a reduction in the values obtained from the lower horizons and would consequently entail an increase in the number of fields needed to be examined in order to obtain a satisfactory measure for these more barren horizons.

The decreased sensitivity of the impression slides (brought about by the higher percentage frequency of mycelium recorded) appears to be due to the fact that, although only soil and mycelium flush with soil surface adheres to the slide, on removal of the slide from the soil surface excess soil and the remainder of the partially adhering mycelium are brought away on the slide. Most of the excess soil may be removed by gently tapping the slide, but this procedure does not remove the "extra mycelium". Consequently the length of mycelium adhering to the slide is longer than would be found if only that part of the mycelium actually flush with the soil surface was brought away on the slide.

With the preparation of soil sections "extra mycelium" is cut away during the grinding procedure, and consequently a much lower percentage of occurrence of mycelium is obtained when using a similar size of sampling area (field).

It has been previously indicated that one of the major difficulties in the use of impression slides for obtaining quantitative measures of mycelium in soil is that regarding the uniformity in the amount of soil adhering on the slides. BROWN (1958) found that the use of a nitrocellulose adhesive mixture overcame any marked variation in the amount of soil collected from sand-dune soils which are low in organic matter content. This was not the case with the soils dealt with in this study — especially in the A₀ H, A₁ and B₁ horizon soils where there was a high organic matter content.

In this investigation it was possible to obtain good replication in weights of soil taken up on impression slides (see Table 3). However when field density data is used to convert such weights to volume it is apparent that there is a marked variation in the amount of soil taken up on the impression slides from the different horizons (Table 3), this being an effect of the different texture of the soil in the different podzol horizons.

Table 3 Comparison of the weight of soil taken up on each of five impression slides from each of the soil horizons

Soil horizon	Weight of soil (gm.)						Total Vol. of soil on 5 slides (cc)
	Slide 1	Slide 2	Slide 3	Slide 4	Slide 5	Mean wt.	
A ₀ H	0.024	0.029	0.020	0.023	0.024	0.024	0.441
A ₁	0.031	0.040	0.045	0.040	0.049	0.041	0.206
A ₂	0.087	0.102	0.085	0.101	0.125	0.100	0.395
B ₁	0.029	0.029	0.033	0.027	0.026	0.029	0.150
B ₂	0.114	0.090	0.116	0.088	0.082	0.098	0.383
C	0.126	0.127	0.137	0.141	0.084	0.123	0.455

Even within the same horizons there was marked variation in the amount of soil taken up on impression slides made at different sampling times (Table 4), this reflecting variations in soil texture within any one horizon.

Table 4 Comparison of the total volume (cc) of soil taken up on 5 replicate impression slides of the six soil horizons at each of six sampling times

Soil horizon	Sampling time					
	July	September	November	January	March	May
A ₀ H	0.441	0.568	0.496	0.705	0.723	0.655
A ₁	0.206	0.441	0.241	0.465	0.478	0.539
A ₂	0.395	0.190	0.488	0.562	0.312	0.400
B ₁	0.150	0.216	0.352	0.307	0.257	0.233
B ₂	0.383	0.374	0.440	0.521	0.450	0.444
C	0.455	0.801	0.543	1.111	0.600	0.678

Although these imperfections in the impression slide technique exist, it is clear that for obtaining a simple comparative assay of fungal mycelium in different soils the measure of percentage frequency of occurrence based on such a technique gives reasonably similar results to those obtained from soil sections. Further more the imperfections of the impression slide technique are outweighed by the fact that the labour involved in preparing impression slides is much less than that for preparing soil sections.

4.2. Density — the estimated number of separate hyphal pieces per cc of soil

It has been previously pointed out that the measure of density (i.e. number of individuals per unit sampling area), although an absolute measure for many soil organisms (e.g. bacteria, protozoa), is a non-absolute measure for soil fungi because of their growth form.

Estimates of the number of separate pieces of hyphae were made in each of three podzol horizons (A_0H , A_1 and A_2 horizons) using soil sections, agar films and soil suspension slides. In each case five replicate slides were examined (200 fields being observed per slide). Table 5 gives the mean estimated number of separate pieces of mycelium per cc of soil in each of the three horizons as obtained by the techniques applied. These data show that the results obtained vary very markedly depending on the type soil preparation observed. Measurements on agar films always gave the highest values, those on soil sections the lowest.

Table 5 Estimated density of hyphal pieces (number/cc soil) in the A_0H , A_1 and A_2 horizons based on observations of soil sections, soil suspension slides and agar films

Soil horizon	Examination technique		
	Soil sections	Soil suspension slides	Agar films
A_0H	172,400	686,600	1,395,000
A_1	113,200	639,500	843,000
A_2	64,100	391,000	672,000

It would appear that the disruption of the soil fabric and the subsequent dilution of the soil involved in the preparation of agar films and soil suspension slides has had the effect of increasing the degree of detectability of hyphal pieces, although it might seem highly probable that the grinding and shaking procedures involved in the disruption of the soil fabric should also cause a certain amount of fragmentation of the mycelium, thereby increasing the density count. No direct evidence of this was forthcoming from this study.

4.3. Estimated length of mycelium, expressed in metres per cc of soil

This measurement may be considered to be a truly absolute quantitative assessment, being totally independent of the method of observation and recording.

Comparative measurements were obtained from soil samples from each of the 6 podzol horizons using the soil sectioning, soil suspension slide, and agar film techniques. The soil samples for this comparison were collected at one sampling time (July, 1961) and from the same exposed soil profile.

Two hundred observations were made on each of five agar films prepared from each soil horizon. After correcting for dilution, moisture content and "field density" the length of mycelium observed on each agar film could be expressed in meters/cc of soil. From these data the mean estimated length of mycelium per cc of soil could be calculated for each horizon (Table 6). Table 6 also gives data obtained from the same number of obser-

vations of soil sections (i.e. 200 observations on each of five soil sections from each horizon).

Table 6 Comparison of the lengths of mycelium (m./cc. soil) in the six podzol horizons, based on observations of agar films and soil sections (July 1961)

Soil horizon	Agar films	Soil sections	Agar films
			Soil sections
A ₀ H	63.74	4.97	12.8
A ₁	43.56	2.95	14.8
A ₂	33.82	2.94	11.4
B ₁	36.59	2.02	18.0
B ₂	1.92	0.74	2.6
C	0.55	0.08	6.4

These data show that estimations of length of mycelium based on soil sections were marked less than the values obtained from observations of agar films. In order to check the validity of this discrepancy as being a result of the difference between the two techniques applied, the comparative experiment was repeated (January, 1962), the data from this is given in Table 7.

Table 7 Comparison of the lengths of mycelium (m./cc. soil) in the six podzol horizons based on observations of agar films and soil sections (January 1962)

Soil horizon	Agar films	Soil sections	Agar films
			Soil sections
A ₀ H	81.64	12.70	6.4
A ₁	81.81	13.45	6.1
A ₂	71.06	11.55	6.2
B ₁	56.11	7.31	7.7
B ₂	40.33	1.23	38.5
C	9.12	0.31	29.4

From both comparative experiments it is clear that the discrepancy factors (indicated by the ratio agar film : soil section) between the measurements of length of mycelium as determined by the two techniques are very large. It was considered that the presence of these large discrepancies warranted a thorough investigation into possible faults and sources of error in both the agar film and soil sectioning techniques.

(a) Check on the agar film technique

Careful consideration of the procedures involved in the preparation of the agar films indicated the possibility of error at three stages in the preparation. These are:

- (i) the possibility that, despite thorough stirring of the soil-water-agar suspension prior to pipetting off small samples, there was an accumulation of mycelium floating on the surface of the suspension;
- (ii) the possibility that the pipetting procedure might allow an accumulation of mycelium at the tip of the pipette, and, since only the first few drops delivered by the pipette were used in agar film production, this would cause an over-estimate of the amount of mycelium present in the suspension;
- (iii) the possibility that, because of the viscosity of the soil-water-agar suspension, agar films of greater thickness than 0.1 mm could be produced.

Data have already been given on these (THOMAS, NICHOLAS and PARKINSON, 1964) and these show that possibilities (i) and (ii) do not occur, and the possibility (iii) can be

eliminated by applying a small square weight (c. 5 g) to the coverslip containing the soil-agar suspension in the haemocytometer slide.

(b) Check on the soil sectioning technique

Consideration of the procedures involved in the preparation of soil sections indicated that there could be no possibility of error in the determination of the amount of soil observed except for the experimental error of the variable thickness of the soil sections. However there still remained the possibility of error due to the method of observation of the soil sections, whereby the observed length of mycelium present in a unit volume of the soil sections was in fact an underestimate of the actual (true) value. Two factors which could bring about such an error were considered possible:

(i) Inaccurate measurement of the length of mycelium actually observed in the soil sections

The measurement of lengths of mycelium involved the projection of the image of the mycelium on to a perspex screen covered with tracing paper. The length was then calculated by running a map-measuring wheel over the line of the image. It is apparent that with such an arrangement it is only possible to record the length of mycelium in a two-dimensional range (i. e. in the horizontal plane parallel to the plane of focus), whereas the mycelium is occurring over a three dimensional range in sections 50 μm thick. Thus, whilst the length of mycelium present in the plane parallel to the plane of focus will be measured accurately (within the limits of observational error), as the plane of mycelium growth moves from the horizontal to the vertical position (with respect to the plane of focus on the section) there will be a gradual percentage reduction in the length of mycelium measured compared with the actual length viewed. No information was collected on the orientation of the mycelium within the soil sections and for the purposes of this argument it must be assumed that mycelium is randomly oriented. On such an assumption the mean angle of orientation of mycelium seen and measured in any soil section will be 45° to the plane of focus. Consequently for a unit length of mycelium actually viewed in a soil section, the length measured will be equal to $\cos 45^\circ$ of the unit length i.e. for every 100 μm of mycelium actually seen the average length measured on the projection system will be only 70.71 μm ($\cos 45^\circ = 0.7071$).

In allowing for this under-estimate in measurement it is necessary to multiply the recorded lengths by a factor of 1.414 (i.e. the reciprocal of $\cos 45^\circ$).

This correction factor only holds true if all pieces of mycelium were under 50 μm in length, since this is the limit in depth of the soil sections. As some pieces observed in this study were over 50 μm long the application of the correction factor ($\times 1.414$) would slightly overcorrect for the error in measurement.

In practice this error of inaccurate measurement is likely to be great because of failure to observe pieces of mycelium which are oriented near to or at right angles of the plane of vision.

(ii) Failure to see all the mycelium present in the soil because of "masking" and observational error

In soil sections the soil is in a relatively undisturbed state and therefore it may be expected that the organic and mineral particles will "mask" from view a certain amount of the mycelium present in the area observed. The application of procedures such as staining and destruction of soil fabric with subsequent dilution of the soil would markedly improve the chance of observing mycelium and would lead to a higher and more accurate estimate of the amount of mycelium in the soil sample. Both these procedures are necessary for the preparation of agar films but are not applied in the preparation of soil sections.

It was considered probable that whilst in agar films the hyaline mycelium would be readily visible due to staining with phenolic aniline blue, it would be more difficult to observe this mycelium in unstained soil sections. NICHOLAS, PARKINSON and BURGESS (1965) showed that hyaline mycelium makes a large contribution to the total length of mycelium in podzol horizons, and, this being so, it was felt that failure to observe all this mycelium could markedly affect the final estimates of total mycelial content of the soils.

In order to check on this possible selectivity against hyaline mycelium in the observation of soil sections in comparison with agar films, a comparative quantitative, differential count of six morphological groups of mycelium was attempted. These groups were

- (1) Short fragments of dematiaceous hyphae.
- (2) Dematiaceous hyphae in situ of growth.
- (3) Thin, hyaline, septate hyphae.
- (4) Broad, aseptate hyaline hyphae.
- (5) Broad, septate, brown-stained hyphae.
- (6) Sparsely septate fragments of purple-black hyphae.

From the July 1961 sampling period sets of five replicate slides (both soil sections and agar film preparations) from the A₀H, A₁ and A₂ horizons were examined, and the length of the six different morphological types estimated in metres per cc of soil. By calculating the mean values (based on five replicate slides) it was possible to determine what percentage of the total length of all mycelium was taken up by each of the six morphological groups. Comparison between this type of data obtained from soil sections and agar films was then possible. The results of this comparative test are given in Table 8.

Table 8 Check on ease of detectability of different morphological groups of mycelium in soil sections and agar films. (Results based on the % of total mycelium in each of the morphological groups 1—6)

Soil horizon	Procedure	Morphological groups of mycelium					
		1	2	3	4	5	6
A ₀ H	Agar films	23.45	21.53	4.09	45.39	2.07	3.47
	Soil sections	22.85	19.90	5.00	38.00	8.73	5.52
A ₁	Agar films	8.00	22.36	7.62	56.99	4.23	0.80
	Soil sections	11.50	16.63	7.06	58.88	4.22	1.71
A ₂	Agar films	5.95	33.44	4.92	52.71	1.83	1.15
	Soil sections	2.25	30.47	14.54	36.81	15.39	0.54

Examination of these data shows that there was generally no marked difference in the relative amounts of the different morphological groups (expressed as a percentage of the total mycelium) as determined from soil sections or agar films. This appears to indicate no differential selectivity against observing hyaline mycelium in soil sections. However this is unlikely since it was only on stained agar films that very fine (c. 0.5 μ m) actinomycete mycelium was observed. The real reason for the similarity in data obtained by both techniques appears to lie in the fact that hyaline mycelium is relatively difficult to observe in both types of preparation. In the agar film technique, although the actinomycete mycelium stained an intense blue, only a relatively low percentage of the hyaline mycelium took up the stain. This is shown in Table 9 in which the percentages of length of mycelium stained in groups 3 and 4 are recorded, together with the calculated percentages of the length of total mycelium stained for each of the three soil horizons studied. The remainder of the so-called hyaline mycelium was stained a light brown colour (presumably by the humic material in the soil) and it appears that this prevented the stain from penetrating to the protoplasm, or the mycelium was inviable and had lost its protoplasm.

Table 9 Percentage of total length of hyaline mycelium (groups 3 and 4) and % of total length of all mycelium stained by phenolic aniline blue in agar film preparations of soil from the A₀H, A₁ and A₂ horizons

Soil horizon	% length of group 3 stained	% length of group 4 stained	% length of total mycelium stained
A ₀ H	26.6	8.2	4.8
A ₁	28.7	11.9	9.0
A ₂	8.9	6.5	3.8

Thus it seems likely that on both soil sections and agar films there would be a greater observational error (an underestimate) with hyaline mycelia than with dematiaceous mycelium. Although on soil sections it would be expected that the observation of hyaline mycelium would be more difficult than on agar films, it appeared (from subjective observation) that other features of this mycelium tend to compensate for this. Length and orientation of the mycelium play a part in its visibility and it appeared that mycelia of groups 3 and 4 were, on average, much greater in length than those of groups 1, 5 and 6. Also they were noticeably more flexuous than groups 1, 2, 5 and 6. These features of increased length and variable orientation of the hyphae within their own length appear to be compensating factors facilitating their detection.

So far it has been shown that the measurements of the length of mycelium present in a unit amount of soil as determined by observation of soil sections and agar film preparations are both subject to error, that of underestimation in the former (due to observation procedure and observational error) and that of overestimation in the latter (due to error in the preparation procedure).

Where possible, these errors have been calculated and the corrected measures of the estimated length of mycelium in meters per cc of soil as obtained by the observation of soil sections and agar films prepared in July 1961 and January 1962 are given in Table 10. The corrected discrepancy factors between the two sets of measures for each horizon at both sampling periods are also given in Table 10.

Table 10 Comparison of corrected estimated lengths of mycelium (m./cc. soil) in the A₀H, A₁, A₂ and B₁ horizons, based on observations of agar films and soil sections prepared from samples taken in July 1961 and January 1962

Soil horizons	July 1961			January 1962		
	Soil sections	Agar films	Agar films Soil sections	Soil sections	Agar films	Agar films Soil sections
A ₀ H	7.02	42.4	6.0	17.85	54.2	3.0
A ₁	4.17	33.4	8.0	19.05	62.5	3.3
A ₂	4.15	30.3	7.3	16.37	63.8	3.9
B ₁	2.86	30.7	10.7	10.36	47.2	4.6

It can be seen that even after the application of the corrections there is still marked discrepancy in the results obtained by the two techniques. In the absence of evidence of further errors accounting for these discrepancies they must be attributed to the general "masking" of mycelium by mineral and organic materials in the soil. This leads to a severe under-estimation in measurements obtained from soil sections, whereas in the preparation of agar films the soil fabric is broken down and the "masking" of mycelium is thereby considerably reduced (and a higher, more accurate estimate of mycelial length obtained).

Table 11 Comparison of the estimated lengths of mycelium (m./cc. soil) in the A₀H, A₁, A₂ and B₁ horizons, based on observations of soil sections, soil suspension slides and agar films prepared from samples taken in July 1961

Horizon	Soil sections	Soil suspension slides	Agar films
A ₀ H	7.02	25.24	42.4
A ₁	4.17	24.41	33.4
A ₂	4.15	19.69	30.3
B ₁	2.86	12.66	30.7

During these comparative tests data were obtained from soil samples collected from the same soil profile. These data are only reasonably, not directly comparable, since it was not possible to apply all techniques to the same soil sample.

From the data given it can be seen that discrepancy factors varied both from one horizon to another at either sampling period, and also within any one horizon from one sampling period to the other. Consequently the discrepancies between measurements of mycelial lengths as obtained in this study from soil sections and agar films may only be taken as a rough indication the comparative value of such soil preparations in allowing the accurate assessment of the total amount of mycelium in the soil.

Soil suspension slides (WIRKAMP, 1960) were prepared from soil taken from the same profile as exposed for the preparation of soil sections and agar films in July 1961. Of the soil suspension slides prepared five were taken for observation (200 observations per slide). From these observations lengths of mycelium were calculated (applying the necessary corrections for dilution, surface area of the smear, moisture content, and "field density"). Table 11 gives data on the mean estimated length of mycelium (m/cc soil) for each of the four horizons studied (A₀H, A₁, A₂ and B₁ horizons) together with the similar data obtained by examination of soil sections and agar films. It can be seen that the data obtained from soil suspension slides was always intermediate between those obtained from soil sections and agar films.

In every case the measurements based on soil suspension slides were always much nearer to the values obtained from the examination of agar films (rather than to the values from soil sections). This gives supporting evidence in favour of the view that disruption of the soil fabric (together with subsequent dilution) increases the likelihood of detecting mycelium in the soil, the degree of disruption involved in the preparation of soil suspension slides being considerably less than that in the preparation of agar films.

5. Synopsis of the values of the different visual examination techniques

5.1. Modified impression slide technique (BROWN, 1958)

These preparations appear to be the most suitable for obtaining a simple comparative assessment of the mycelium concentration in different soils (in terms of the percentage frequency of occurrence of mycelium in sampling fields of known size). Although it is apparent that such preparations suffer from inaccuracies, due to the variability in amount of soil taken up on the slides, the speed and simplicity of application out-weighed its errors when the technique is used for rough comparative assessments. BROWN (1958) pointed out that in soils of low organic content and reasonably similar texture the extent of these errors will be considerably reduced.

5.2. Agar film technique (JONES and MOLLISON, 1948)

It is apparent from this study that the agar film technique provided the best method for accurate, absolute, quantitative assessments of amounts of mycelium in soil. Modifications reducing the possibility of error using this technique have been described (THOMAS, NICHOLAS and PARKINSON, 1964).

The one major drawback with regard to this technique appears to lie in the fact that the larger mineral particles of the soil sample are not taken into the suspension. Consequently, as BROWN

(1958) pointed out, it is possible that mycelium closely associated with this material would be lost to the prepared films. Observation of soil remaining in the mortar after preparation of the suspensions does reveal the presence of mycelium but quite a large proportion of this appears as free mycelium not directly associated with larger soil particles. This retention would undoubtedly result in an underestimate of amounts of mycelium but this error could be considerably reduced by lowering the concentration of soil for the preparation of agar films.

5.3. Soil suspension slide technique (WITKAMP, 1960)

Assessment of concentration of mycelium obtained by this technique were very similar, but lower, to those afforded by agar films.

The one major advantage of the soil suspension slide technique over the agar film technique appears to be that it permits larger mineral particles of the soil to be taken up into the soil preparations. This could be of value in studies on soils where a large proportion of the mycelium is closely associated with such material. However, in this study, no evidence was obtained to indicate the possibility of an improved measure due to this factor.

5.4. Soil sectioning technique (NICHOLAS and BURGESS, 1961)

For absolute, quantitative assessments of mycelium per unit amount of soil this technique is markedly inferior to the agar film of soil suspension slide techniques. The reasons for this have been previously discussed.

Although the application of the soil sectioning technique provided gross under-estimates of the amounts of mycelium present in soil samples, it must be remembered that the major advantage of the technique lies in the fact that it allows the detail examination of relatively undisturbed soil under high magnification.

If the soil sectioning technique is to be used to its best advantage it should be applied to problems requiring data on the patterns of growth of soil fungi and the association of these micro-organisms to the varied soil microhabitats (i.e. studies of fungi "in situ" in the soil).

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